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RAPID GENERATION OF RECOMBINANT ADENOVIRAL VECTORS

Clean Set of Pending Claims

- The cloning system of claim 16, wherein any or all open reading frames constituting E4 2. have been modified in the backbone plasmid.
- The cloning system of claim 2, wherein the modification is a substitution, insertion, or 3. deletion of one or more nucleotides.
- The cloning system of claim 16, wherein E3 has been modified in the backbone plasmid. 4.
- The cloning system of claim 4, wherein the modification is a substitution, insertion, or 5. deletion of one or more nucleotides.
- The cloning system of claim 4, wherein E3 has been modified to contain a multiple 6. cloning site.
- The cloning system of claim 4, wherein one or more genes required for Herpes Simplex 7. Virus (HSV) packaging and an HSV origin of replication have been placed within the E3 region.
- The cloning system of claim 16, further comprising in the backbone plasmid HSV 8. Amplicon sequences required for packaging and replication.
- The cloning system of claim 16, further comprising in the backbone plasmid one or more 10. sequences that allow for integration of sequences into cells after viral infection.
- (Amended) A shuttle plasmid consisting essentially of Ad sequences from 0 to 1 and 9.2 11. to 16.1 map units of an Ad genome when the shuttle plasmid lacks a loxP sequence.



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- 12. The shuttle plasmid of claim 11, wherein PacI restriction endonuclease sites flank either end of the Ad sequences.
- 13. The shuttle plasmid of claim 11, further comprising a multiple cloning site positioned between 1 and 9.2 map units.
- 14. The shuttle plasmid of claim 11, wherein the shuttle plasmid further comprises a sequence encoding a gene of interest.
- 15. (Amended) The shuttle plasmid of claim 11, further comprising a promoter, or other sequence used to drive expression from a transgene.
- 16. (Amended) A cloning system for generating recombinant adenovirus comprising:
 - (a) an Ad backbone plasmid consisting essentially of an Ad genome lacking map units 0 to 9.2, wherein the numbering of the map units starts with the lefthand ITR and wherein the backbone plasmid lacks a loxP sequence, and
 - (b) a shuttle plasmid consisting essentially of Ad sequences from 0 to 1 map units and 9.2 to 16.1 map units of an Ad genome wherein the shuttle plasmid lacks a loxP sequence.
- 17. (Amended) A host cell comprising
 - (a) an Ad backbone plasmid comprising an Ad genome lacking map units 0 to 9.2, wherein the numbering of the map units starts with the lefthand ITR and wherein the backbone plasmid lacks a loxP sequence, and
 - (b) a shuttle plasmid comprising Ad sequences from 0 to 1 map units and 9.2 to 16.1 map units of an Ad genome wherein the shuttle plasmid lacks a loxP sequence.
- 18. The host cell of claim 17, wherein the cell expresses E1 sequences necessary for supporting adenovirus replication.

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- 19. A host cell of claim 18, wherein the cell is an animal cell.
- 20. A host cell of claim 17, wherein the cell expresses E1 sequences, pIX and E4 sequences required for amplification of viruses generated made with the Ad backbone lacking E1, E1 and pIX, or E1 and E4, respectively.
- 21. A host cell of 20, wherein the cell is an animal cell.
- 22. (Amended) A method for producing recombinant adenovirus comprising contacting a host cell with
 - (a) an Ad backbone plasmid comprising an Ad genome lacking map units 0 to 9.2, wherein the numbering of the map units starts with the lefthand ITR and wherein the backbone plasmid lacks a loxP sequence, and
 - (b) a shuttle plasmid comprising Ad sequences from 0 to 1 map units and 9.2 to 16.1 map units of an Ad genome wherein the shuttle plasmid lacks a loxP sequence.
- 23. The method of claim 22, further comprising serially amplifying virus produced by the host cell.
- 24. The method of claim 23, further comprising detecting the presence of wild type virus.
- 25. The method of claim 22, wherein the shuttle plasmid further comprises a sequence encoding a gene of interest.